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Arthur L. Weber, Principal Investigator

NASA Grant NCC 2-784 · NASA Task 185-52-22-14

Progress Report

August 1, 1994 - July 31, 1995

(NASA-CR-199062) REDOX ENERGY AND
SULFUR CHEMISTRY IN PREBIOTIC
POLYMER SYNTHESIS AND REPLICATION
Progress Report, 1 Aug. 1994 - 31
Jul. 1995 (Search for
Extraterrestrial Intelligence
Inst.) 6 p

N95-71563

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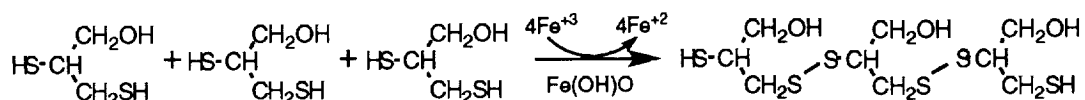
Redox Energy and Sulfur Chemistry in Prebiotic Polymer Synthesis and Replication

Arthur L. Weber, Principal Investigator

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Report summary: In the past three years we have made significant progress in four research areas; 1) We developed a new model of prebiotic polymer synthesis that yields polydisulfide polymers when dithiols are oxidized by iron(III) of synthetic minerals. 2) Our calculations of average oxidation number of the biocarbon of several types of organisms showed that the biosphere is very close to the reduction level of formaldehyde (oxidation number = 0). 3) Our estimates of the free energy of formation of biochemicals from one-carbon substrates revealed that their energy of formation is determined mainly by the oxidation state of the substrate and the number of carbons in the product. These energy values can be used to predict the plausibility of prebiotic synthetic reactions. 4) We calculated the free energy and the carbon redox disproportionation values for (a) 25 fermentation reactions, and (b) the biosynthesis of the amino acids, lipids, and nucleotides from glucose. We found that free energy of the fermentation reactions was directly proportional to the degree of redox disproportionation of carbon, and that the biosynthesis of amino acids and lipids was driven by the redox disproportionation of glucose that yielded a large amount of useful chemical energy. We concluded that the redox disproportionation of carbon was the primary energy source of amino acid and lipid biosynthesis from glucose. We also spent considerable time moving our laboratory from the Salk Institute and establishing it on site at the Ames Research Center.

Prebiotic oxidative polymer synthesis (Weber 1995a). A major problem in the prebiotic synthesis of anhydride polymers like polypeptides and polynucleotides is the destruction of chemical condensing agents and reactive intermediates by water that prevents the formation of large polymers. As a way to overcome this problem we examined the prebiotic oxidative polymerization of a dithiol (2,3-dimercaptopropanol) by ferric ions on the surface of iron(III) hydroxide oxide [Fe(OH)O]. The oxidative synthesis of the trimer is shown below. Polydisulfide polymers up to 15 units long were synthesized from low concentrations of dithiol monomer (1mM) under mild conditions (pH 4-6, 40°C, 3 days). Synthetic goethite (α -Fe(OH)O) and synthetic magnetite (Fe_3O_4) gave similar oligomer yields.



The oxidative polymerization of 2,3-dimercaptopropanol is an attractive prebiotic reaction because the reaction 1) needs only a small three-carbon monomer, 2) occurs readily at low monomer concentration to give reasonably large oligomers (up to 15-mer) under mild conditions, and 3) takes place without chemical interference by water. Ferric ions needed for the formation of Fe(OH)O and Fe_3O_4 on the primitive Earth could have been continually generated by photooxidation of dissolved ferrous ions.

Estimation of the reduction level of biocarbon (Weber 1995b). In order to better understand metabolism we calculated the average oxidation number of biocarbon of several types of organisms from their biochemical composition and the average oxidation number of their molecular constituents (protein, lipid, polysaccharide, and nucleic acid). Figure 1 below shows the oxidation number (oxid. #) of carbon functional groups. Carbon dioxide with an oxidation number of +4 is the most oxidized form of carbon, and methane with an oxidation number of -4 is the most reduced form of carbon. We found that the reduction level of most plants and microbes was between +0.04 and -0.22. Since over 99% of the carbon of the biosphere resides in microbes and land plants (90% in forests), our calculations established that the average oxidation number of the carbon of the Earth's biosphere was near 0.10 -- a value near the reduction level of formaldehyde (oxid. # = 0.0) which requires 4 electrons to be synthesized from carbon dioxide. Moreover, we found that if the carbon dioxide by-product of amino acid and lipid biosynthesis was included in the calculation, the average reduction level of biosynthetic products of every organism in our sample was very close to that of formaldehyde (oxid. # = 0.0).

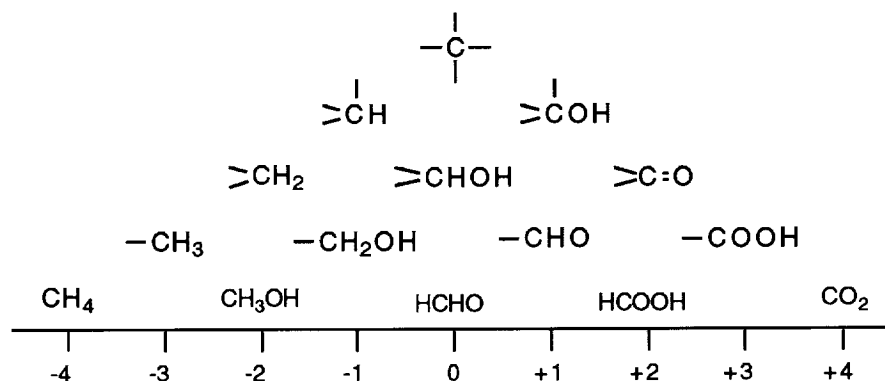


Fig.1. Oxidation numbers of carbon groups are given directly below each group. Open bonds are to other carbons and can be saturated or unsaturated. O can be replaced by N or S.

Estimation of the free energy of formation of biochemicals (manuscript in preparation for J. Mol. Evol.). We also calculated the standard free energy (ΔG°) of formation of amino acids, nucleosides, sugars, and fatty acids from simple one-carbon precursors. These energy values can be used to estimate the plausibility of prebiotic reactions, because they indicate the degree to which reactants or products are favored in a reaction at equilibrium under standard conditions. Negative energy values indicate that the product formation is energetically favorable; whereas, positive energy values indicate that product formation is unfavorable. Values were calculated for the synthesis of the 20 protein amino acids and the 12 amino acids from spark discharge experiments using one carbon precursors (carbon dioxide, formic acid, formaldehyde, methanol, or methane) and inorganic nitrogen (NH_3 or N_2). Water acted as a source of hydrogen or oxygen. Values were also calculated for carbon dioxide reactions using other reductants (H_2S , Fe^{+3} , or H_2). The energy values of these nearly 300 reactions were calculated using Mavrovouniotis's method to estimate the standard free energy of formation of reactants and products.

As depicted in Figure 2 below we discovered that the synthesis of amino acids from methane as a carbon source became energetically more unfavorable as the number of carbons in the amino acid increased. This dependence of energy on size is probably responsible for the 100-fold decrease in the yield of spark discharge amino acids going from the 2-carbon glycine ($\Delta G^\circ \sim 50$ kcal/mol) to the 6-carbon amino acids ($\Delta G^\circ \sim 130$ kcal/mol) - the largest amino acids detected. The apparent energy limit of 130 kcal/mol for synthesizing a detectable amount of an amino acid suggests that substances having higher positive energy values (like adenosine requiring 350 kcal/mol) would not be synthesized in detectable yields in similar spark discharge reactions. We also concluded from the data in Figure 3 that amino acid synthesis was most favorable when the reduction level of the carbon of the substrate matched that of the amino acid product.

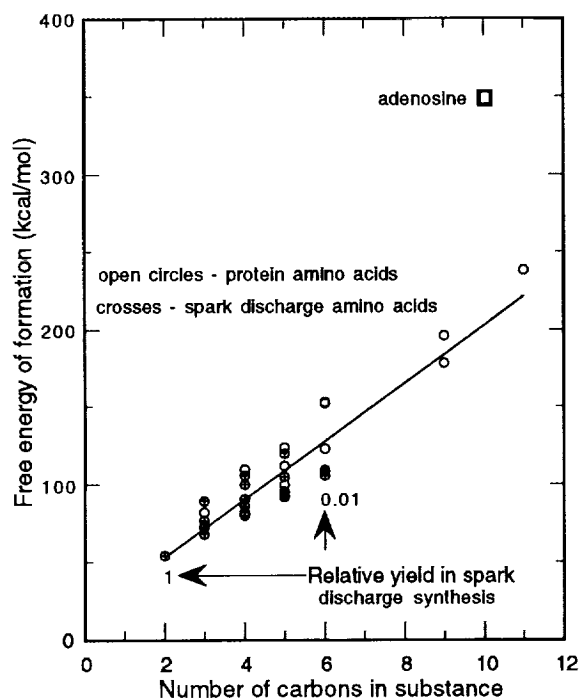


Fig. 2. Free energy of formation of amino acids and nucleosides from CH_4 , NH_3 , and H_2O as a function the number of carbons of each substance.

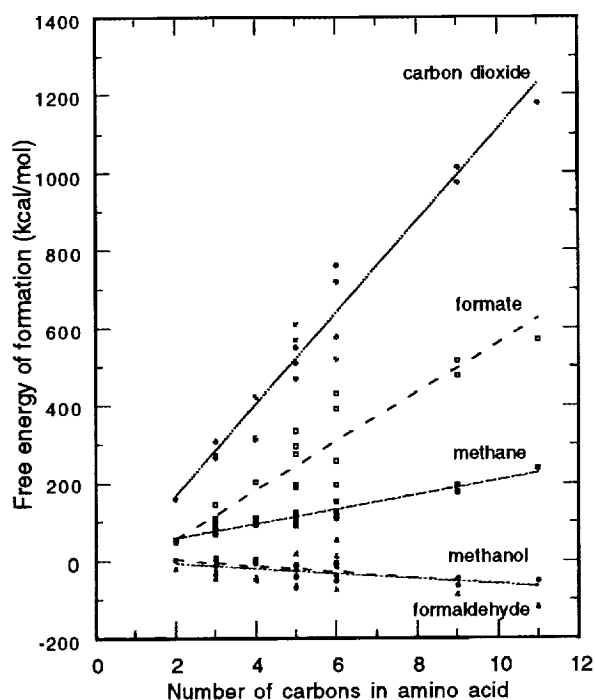


Fig. 3. Free energy of formation of protein amino acids from one carbon substrates, NH_3 , and H_2O as a function of the number of carbons of each amino acid.

Redox disproportionation in fermentation and biosynthesis (manuscript in preparation for Nature). In contemporary life one-carbon substrates are converted to formaldehyde or its adducts (sugar groups and serine's hydroxymethyl group) before they enter biosynthetic metabolism. This observation that carbon enters biosynthesis at the reduction level of formaldehyde (oxid. # = 0.0), together with our earlier finding that the products of biosynthesis also have an average oxidation number near 0.0, indicates that biosynthesis is primarily a redox disproportionation process where electrons are exchanged between carbons without changing the average reduction level (oxid. #) of the processed carbon. This observation raised the question: why does biosynthesis start with carbon at the formaldehyde reduction level and then proceed by disproportionation? In order to answer this question, we calculated the Gibbs free energy change of (a) the 25 carbon fermentation reactions and (b) the biosynthesis of *E. coli*'s amino acids,

lipids, and nucleotides from glucose. We also calculated the degree of redox disproportionation of carbon of these reactions. The redox disproportionation of a reaction was defined as the sum of the absolute values of the change in the oxidation number of all carbon in a reaction. As shown in Figure 4 below, we found that the energy yield ($-\Delta G/\text{carbon}$) of the fermentation reactions (numbered 1-25) was directly proportional to the degree of redox disproportionation ($\Delta DP/\text{carbon}$). Figure 4 also shows that in *E. coli* amino acid and lipid biosynthesis are essentially fermentative processes that are driven by energy from the redox disproportionation of glucose carbon. Although amino acid and lipid biosynthesis yield roughly as much ATP as they consume, nucleotide biosynthesis which is not driven by redox disproportionation relies completely on exogenous ATP for energy. Moreover, the favorable energetics of redox disproportionation is established by the chemistry of carbon that sets the direction of electron flow in favor of disproportionation. As shown in Figure 5 the overall direction of electron transfer (indicated by the arrows) is from more oxidized carbon groups that are stronger reductants (reduction potentials are more negative) to less oxidized carbon groups to give reduction products that are weaker reductants (reduction potentials are less negative).

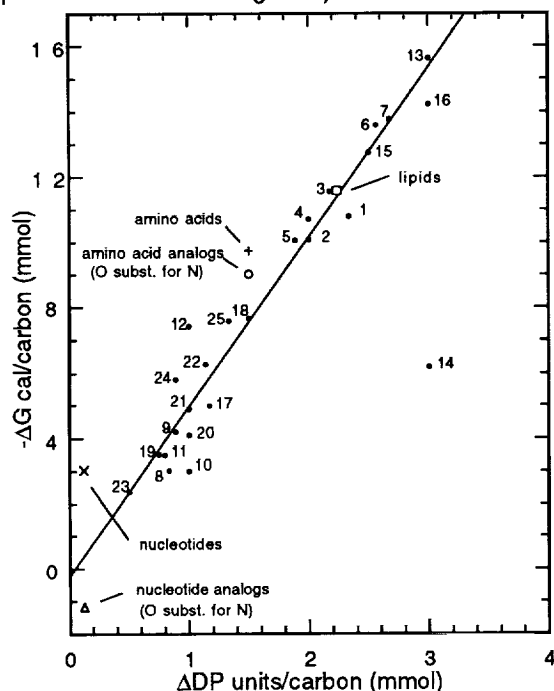


Fig. 4. Free energy change ($-\Delta G$) per mmol carbon plotted as a function of the redox disproportionation (ΔDP) per mmol carbon of 25 fermentations and 3 biosynthetic processes.

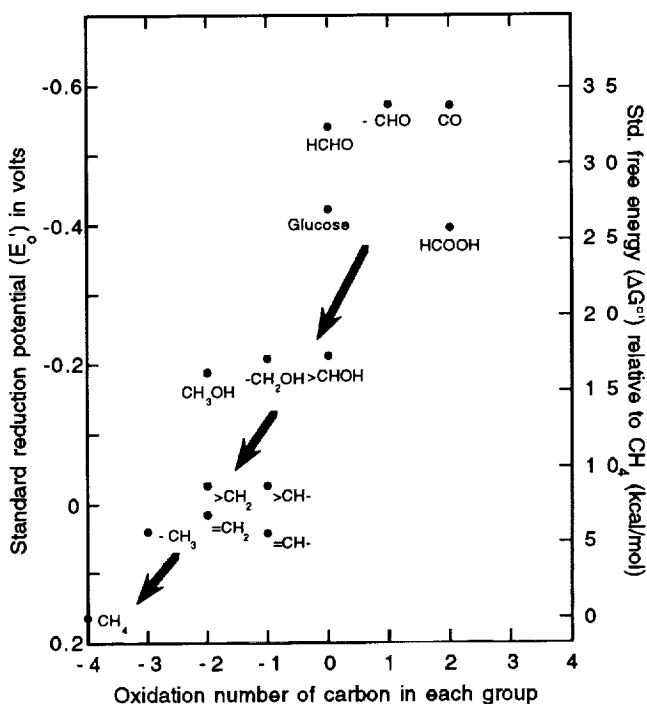
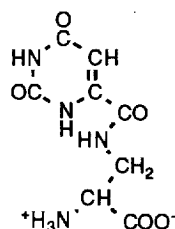


Fig. 5. Standard reduction potential of groups plotted of their carbon oxidation number.

We concluded that biosynthetic transformations are primarily driven by and depend on the chemical energy made available by the redox disproportionation of carbon groups at the formaldehyde (or sugar) level of reduction. Since this result suggested that the disproportionation of carbon at the formaldehyde reduction level was a universal biological energy source, we proposed that this shared property of life in the Universe could lead to the selection of a microwave frequency of formaldehyde (like the 72.8 GHz line of formaldehyde's $0,0,0 \rightarrow 1,1,1$ rotational transition) as an interstellar contact channel.

Chemical self-replication by polypeptides. We have begun to synthesize amino acids having nucleotide-like side chains that will be used to prepare nucleotide-like polypeptides for studies of polypeptide self-replication. So far we have synthesized the 'nucleotide' amino acid, N- β -orotidyl-L-diaminopropionic acid shown below. N- β -Orotidyl-L-diaminopropionic acid was synthesized by using 1,1'-carbonyldiimidazole to couple orotic acid and N- α -Boc-L-diaminopropionic acid and employing trifluoroacetic acid to remove the Boc protecting group. We are now developing techniques to obtain large amounts of this 'nucleotide' amino acid and its Boc-protected derivative in the pure state. Later polypeptides with nucleotide-like side chains will be prepared using these 'nucleotide' amino acids.



N- β -orotidyl-L-diaminopropionic acid

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